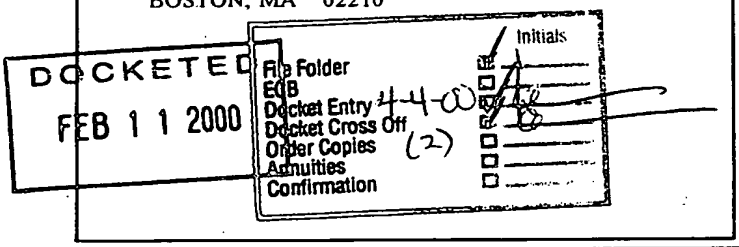
PATENT COOPERATION TREATY

IFD

From the INTERNATIONAL SEARCHING AUTHORITY

To: WILLIAM G. GOSZ
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210



PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing *(day/month/year)* 04 FEB 2000
Applicant's or agent's file reference G0651/7000WO	**FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US99/23014	International filing date *(day/month/year)* 01 OCTOBER 1999
Applicant GENZYME CORPORATION	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer AMY DECLOUX
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

The statement should be brief, it should not exceed 500 words if in English or if translated into English.

It should not be confounded with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It should not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

In what language ?

The amendments must be made in the language in which the international application is published. The letter and any statement accompanying the amendments must be in the same language as the international application if that language is English or French; otherwise, it must be in English or French, at the choice of the applicant.

Consequence if a demand for international preliminary examination has already been filed ?

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase ?

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

NOTES TO FORM PCT/ISA/220

These Notes are intended t give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty and of the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed informati n, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended ?

The claims only.

The description and the drawings may only be amended during international preliminary examination under Chapter II.

When ? **Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).**

Where not to file the amendments ?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How ? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

What documents must/may accompany the amendments ?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confounded with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

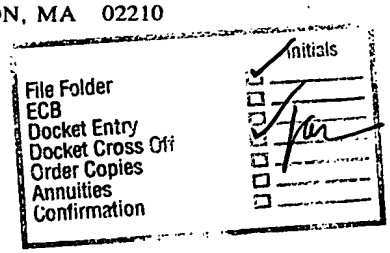
The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;
(ii) the claim is cancelled;
(iii) the claim is new;
(iv) the claim replaces one or more claims as filed;
(v) the claim is the result of the division of a claim as filed.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: WILLIAM G. GOSZ
WOLF, GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210



PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)* 19 JAN 2001	
Applicant's or agent's file reference G0651/7000WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/23014	01 OCTOBER 1999	02 OCTOBER 1998

Applicant
GENZYME CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	David Saunders TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992) ★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference G0651/7000WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/23014	International filing date *(day/month/year)* 01 OCTOBER 1999	Priority date *(day/month/year)* 02 OCTOBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant GENZYME CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 26 APRIL 2000	Date of completion of this report 04 DECEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer David Saunders Telephone No. (703) 308-0196

TERRY J. DEY
PARALEGAL SPECIALIST
TECHNOLOGY CENTER 1600

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/23014

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-8, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 9, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages 1-6, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-3, 5 and 7-10	YES
	Claims	4 and 6	NO
Inventive Step (IS)	Claims	10	YES
	Claims	1-9	NO
Industrial Applicability (IA)	Claims	1-10	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 4 and 6 lack novelty under PCT Article 33(2) as being anticipated by Richards et al. Richards et al teach an antibody to TIMP-1 (see entire document, especially column 14. lines 23-26). Therefore, the referenced teachings anticipate the claimed invention.

Claims 1-3, 5 and 7-9 lack an inventive step under PCT Article 33(3) as being obvious over Richards et al in view of Sterns et al and Menino, Jr. et al. Richards et al teach an antibody to TIMP-1 (see entire document, especially column 14. lines 23-26). Richards et al also teach that cells transformed with DNA encoding antisense TIMP-1 RNA were down regulated for TIMP-1 production (see entire article including column 4, lines 42-61.) Richards et al. also teach that wound healing and remodeling are also affected by metalloproteinase activity (see column 3, lines 35-45) and that regulation of TIMP activity is important to promote the healing of injury (see column 6, lines 7-14), and that therapeutic regulation of metalloproteinases can be accomplished by altering the level of TIMP in a patient (see column 6 lines 56-67).

Stearns et al teach TIMP-1 antisense probes (see entire article, including page 178 legend of Figure 5). Menino, Jr. et al also teaches primers to TIMP-1 including antisense nucleotides (see entire article, especially, Table 1).

Therefore it would have been obvious to one of skill in the art at the time the invention was made, to use antibodies to TIMP-1 or TIMP-1 antisense oligonucleotides to inhibit or control TIMP activity because TIMP is taught by Richards et al to be important in tissue remodeling which occurs during the formation of surgical adhesions. In view of the standard molecular techniques of making monoclonal antibodies at the time the invention was made, it would have been obvious to make a monoclonal as well as a polyclonal antibody to TIMP-1, and to make a composition thereof which includes the common carrier hyaluronic acid, especially in view of the teachings of an antibody to TIMP-1 by Richards et al. and his teachings of its potential therapeutic (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C12Q 1/68; G01N 33/53; C07K 16/44 and US Cl.: 424/130.1, 145.1; 435/6, 7.1; 436/547; 530/387.1, 388.25

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
use.

Claim 10 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of determining whether a human subject is predisposed to develop adhesions following surgery by measuring TIMP levels.

Claims 1-10 meet the criteria set out in PCT Article 33(4), for industrial applicability.

---------- NEW CITATIONS ----------
NONE

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
GOSZ, William, G.
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, MA 02210
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 13 April 2000 (13.04.00)	
Applicant's or agent's file reference G0651/7000WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US99/23014	01 October 1999 (01.10.99)	02 October 1998 (02.10.98)

Applicant
GENZYME CORPORATION et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   13 April 2000 (13.04.00) under No. WO 00/20642

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68, G01N 33/53, C07K 16/44

A1

(11) International Publication Number: **WO 00/20642**

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/23014

(22) International Filing Date: 1 October 1999 (01.10.99)

(30) Priority Data:
60/102,869 2 October 1998 (02.10.98) US

(71) Applicants *(for all designated States except US)*: GENZYME CORPORATION [US/US]; Metrowest Place (MWP), P.O. Box 9322, Framingham, MA 01701–9322 (US). UNIVERSITY OF FLORIDA [US/US]; 1938 W. University Avenue, Gainesville, FL 32603 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHEGINI, Nasser [US/US]; 6813 N.W. 90th Street, Gainesville, FL 32650 (US). BURNS, James [US/US]; 182 Standish Road, Watertown, MA 04122 (US). DIAMOND, Michael [US/US]; 45 Oxford Road, Grosse Pointe, MI 48236 (US). HOLMDAHL, Lena [SE/SE]; Sofiehojdsvagen 2C, S–441 43 Alingsas (SE).

(74) Agent: GOSZ, William, G.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PREVENTION OF ADHESIONS

(57) Abstract

Methods for the prevention of adhesion formation involve the administration of therapeutic formulations to a patient which include antibodies to TIMP–1 or TIMP–1 antisense oligonucleotides. The formulations can also include suitable carriers, such as a hyaluronic acid matrix, for optimal administration. The treatment procedure can be initiated and monitored by a diagnostic procedure which involves the detection of elevated levels of TIMP–1 in a patient.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
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CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
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CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation.		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PREVENTION OF ADHESIONS

Background of the Invention

It is well established that post-operative adhesions develop in the vast majority of patients after surgery. Injury or inflammation in the peritoneal cavity produces a fibrous exudate. As a result, the serosal surfaces stick together. The fibrous exudate may be absorbed or invaded by fibroblasts to form a permanent fibrous adhesion.

Removal of fibrin before it is invaded by fibroblasts prevents the formation of permanent fibrous adhesions. Removal of fibrin occurs due to the fibrinolytic activity of the peritoneal cavity. Fibrinolytic activity can vary as a result of surgery. Fibrinolytic activity is absent from a peritoneal wound during the first 48 hours after surgery. However, there is a gradual increase after this time up to 8 days when the peritoneum heals. The source of the fibinolytic activity is found in the mesothelial cells. It is postulated that the absence of definitive mesothelial cells with their associated fibrinolytic activity may facilitate adhesion formation by allowing fibroplasm to occur before definitive mesothelial cells have grown between and separated the two opposed surfaces of a fibrinous adhesion.

The molecular events underlying peritoneal wound healing and development of fibrous adhesions are complex and multifactoral. The cascade of events that leads to peritoneal wound repair in many aspects resembles those that occur during skin wound healing, which is characterized by inflammation, cellular migration, proliferation, phenotypic differentiation and tissue remodeling. Tissue remodeling involves deposition and degradation of the extracellular matrix, which are highly regulated processes, occurs throughout wound repair, and are influenced by a host of locally expressed growth factors, cytokines and eicosanoids. The extracellular matrix is a dynamic component capable of modulating various cellular activities including cell-cell interaction, proliferation, differentiation and sequestering potent biological response modifiers from the wound environment. In addition, it has become clear that excess production and deposition of the extracellular matrix is a key factor in producing tissue fibrosis throughout the body including the development of peritoneal adhesions.

It has been suggested that serine proteases and metalloproteinases not only play a critical role in various stages of normal wound repair, but are involved in enhanced breakdown of the major components of the extracellular matrix in pathological wound healing. Matrix metalloproteinases ("MMPs") are members of a family of zinc proteases which hydrolyze various components of the extracellular matrix such as collagens, fibronectin, laminin, elastin and protoglycans. Seventeen different MMPs have been isolated and characterized, which based on their substrate specificity are divided into several subgroups: collagenases (MMP-1, MMP-8, MMP-13), gelatineses (MMP-2 and MMP-9), stromalysins (MMP-3, MMP-7, MMP-10, MMP-11), matrilysins (MMP-9), and the newly discovered membrane-type MMPs (MT-MMP1 to MT-MMP-4 or MMP-14 to MMP-17). The catalytic activity of MMPs is regulated at least in part by a group of proteins referred to as tissue inhibitors of matrix metalloproteineases or TIMPs. Four TIMPs have been identified and are referred to as TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

A coordinated expression and balance between the production of MMPs and TIMPs is an important step in tissue remodeling. In general, MMPs are not expressed constitutively *in vivo* in adult tissues, but they are induced in response to various stimuli including proinflammatory cytokines, growth factors and hormones. MMPs are also induced in tissues that normally undergo extensive remodeling such as the endometrium during the menstrual cycle and wounds during healing. Furthermore, an important feature of the MMPs is that they are produced as inactive proenzymes and require activation, which is achieved by various factors including several serine proteineases such as plasmin, trypsin and neutrophil elastase. In contrast, the expression of TIMPs is wide spread in many tissues and is regulated in co-ordination with MMPs. TIMP-1 and TIMP-2 inhibit the activity of all MMPs by forming a high affinity complex in a 1:1 ratio. In addition to inhibiting the MMPs activity, TIMPs have also been shown to have growth factor like activity by stimulating cell growth.

Thus, for normal peritoneal healing to occur, the availability of these molecules must be optimal, precise, and synchronized. Inhibition, interruption, or excess expression of these molecules seems to be responsible for failure in normal healing, resulting in either impairment or excess tissue formation (adhesion development). Although the role of growth factors, cytokines, eicosanoids and serine proteinases have been investigated in relation to peritoneal wound repair and adhesion formation, there is no information currently available in respect to the expression of MMPs and TIMPs in the peritoneal environment.

The formation of intraperitoneal fibrous adhesions is a complex process that involves migration and mitosis of a variety of cell types, including inflammatory cells, mesothelial cells, and fibroblasts. Peptide growth factors and their receptors may play key roles in regulating many aspects of adhesion formation. Growth factors, such as epidermal growth factor (EGF), and transforming growth factor-β (TGF-β) may directly influence adhesion formation.

Summary of the Invention

It has now been discovered that an unbalanced level of MMP-1 and TIMP-1 in a human subject, high TIMP-1 expression, and the association of a major portion of MMP-1 in complex with TIMP-1 may be major contributing factors in the peritoneal environment by providing a favorable condition for adhesion development. This discovery has lead to the development of novel methods for treating surgical adhesions, for diagnosing the probability of developing adhesion formation, and for preparing pharmaceutical formulations for reducing or preventing adhesions.

In one particular aspect of the invention, a method for the prevention or remediation of surgical adhesions comprises treating a patient at risk of having such adhesions with a therapeutic formulation selected form the group consisting of antibodies to TIMP-1 and TIMP-1 antisense oligonucleotides. Treatment with TIMP-1 antibodies results in the alteration of local levels of both TIMP-1 and MMP. Antisense oligonucleotides can be targeted to a specific gene's mRNA destruction to inhibit the synthesis of proteins.

In another aspect of this invention, antibodies to TIMP-1 are disclosed and used to formulate a therapeutic formulation for the treatment or prevention of surgical adhesions. The antibodies can be polyclonal antibodies, monoclonal antibodies or Fab fragments. The formulation can include suitable carriers and adjuvants. A particularly preferred carrier is a hyaluronic acid matrix, which can be derivatized, underivatized or cross-linked.

An additional aspect of this invention involves a method for the detection of a predisposition in a subject to adhesion formation which comprises the detection of elevated levels of TIMP-1 in a human subject. Once detected, the predisposition for adhesion formation can then be treated using the procedure of this invention.

Brief Description of the Drawings

Figure 1 is a diagram showing the expression of MMP-1 in intraperitoneal tissue.

Figure 2 is a diagram showing the expression of TIMP-1 in intraperitoneal tissue.

Figure 3 is a diagram showing the co-expression of MMP-1 and TIMP-1 in intraperitoneal tissue.

Figure 4 is a diagram showing MMP-1 production levels.

Figure 5 is a diagram showing the comparative levels of TIMP-1 production in mild as compared to extensive adhesions.

Figure 6 is a diagram showing the comparative levels of TIMP-1 expression in the female peritoneal environment for both pre-menopausal and post-menopausal women.

Detailed Description of the Invention

An important area for the prevention of adhesion formation is the modulation of growth factors and cytokines. The present invention provides for the first time a comparative analysis of the level of expression of MMP-1, TIMP-1 and MMP-1/TIMP-1 in various tissues within the peritoneal cavity and peritoneal fluids of patients who were undergoing pelvic/abdominal surgical procedures. The results indicate that interstitial collagenase or MMP-1, which degrades type I, II, II and VII collagens, is expressed at a significantly higher level in ovaries and fallopian tubes compared to skin, fascia, parietal peritoneum, omentum, uterus, and large bowl, as well as fibrous adhesions, with lowest levels associated with skin. Also, in peritoneal fluid the level of MMP-1 is low and comparable to that detected in skin, which under normal conditions expresses low to undetectable levels of MMPs. Comparatively, adhesions express a moderate level of MMP-1, which is significantly lower than in ovaries and higher than in skin.

In contrast to MMP-1, the expression of TIMP-1 in tissues was highest in adhesions with ranges from 2 to 8 fold higher, but approximately 1.5 fold lower than that detected in peritoneal fluid. These results suggest that in the peritoneal environment, tissues such as ovaries, fallopian tubes and uterus express higher levels of MMP-1 and TIMP-1. The results are consistent without regard to the cause of trauma, i.e. ovaries, fallopian tubes and uterus appear to be more susceptible to adhesion formation following trauma, irrespective of whether it is caused by physical, cytotoxic, inflammatory or immunological factors. This may be due to high levels of TIMP-1 expression which inactivates all the MMPs including MMP-1 by forming complexes with TIMPs in a 1:1 ratio.

In support of this conclusion, we observed that in patients with extensive adhesions, the level of TIMP-1 expression was substantially higher than those with moderate or mild adhesions. Although there appears to be a trend for higher TIMP-1 expression in patients with extensive adhesions, due to variability in the number of patients within each group and inconsistency in the type of tissues collected during sampling, it is difficult to reach a conclusion at the present time regarding the levels in patients with and without adhesions. The results further indicate that the level of MMP-1/TIMP-1 complex in the ovaries and uterus is the highest, compared to other tissues, and corresponds to 55 to 70% of total MMP-1 level expressed in these tissues. Such a relationship between the level of MMP-1/TIMP-1 complex, and the level of total MMP-1 was also observed in other tissues, with levels ranging from 37% to 69%.

Peritoneal fluid is also regarded to play a key role in development of adhesion formation, due to the presence of various factors. With regard to the peritoneal fluid, MMP-1and MMP-1 /TIMP-1 complex was low compared to their tissue levels. However, peritoneal fluid contained the highest level of TIMP-1. Furthermore, the adhesions also express a low level of MMP-1 and MMP-1/TIMP-1 complex, while they expressed the second highest level of TIMP-1 compared to other tissues. It would appear that 100% of total MMP-1 detected in peritoneal fluids and 65% in the adhesions was in complex with TIMP-1. This suggests that the role of peritoneal fluid in the context of adhesion formation favors matrix deposition rather than degradation, and is consistent with the clinical impression. Thus, once an adhesion develops, it will persist and does not spontaneously resolve. Furthermore, this milieu favors extracellular matrix deposition, and is consistent with clinical reports that adhesions become thicker and more dense over time. Although the adhesions examined in this report are mature and far less dynamic, our data suggest that they appear to exist under a molecular environment which prevents proteolytic enzyme degradation by MMPs. Furthermore, in addition to inhibiting the activity of the MMPs, TIMP-1 has been demonstrated to have growth factor like activity by stimulating cell growth. Because of the high content of TIMP-1 in the peritoneal fluid, TIMP-1 may have a stimulatory effect on cell growth, including fibroblasts which migrate into the site of injury at the initial stage of adhesion formation.

Potentially, several growth factors, cytokines and eicosanoids, which are expressed by these tissues and present in the peritoneal fluid, can regulate the expression of MMPs and TIMPs. In addition, in tissues such as the uterus and the ovary, the expression of MMPs and TIMPs has been shown to be regulated by ovarian steroids and gonadotropins, respectively. In this respect,

MMPs have been associated with endometrial breakdown during the menstrual cycle and progesterone has been reported to inhibit the expression of a selective number of MMPs in this tissue. Among the growth factors and cytokines, it is well established that excess production of TGF-β in various tissues leads to pathological fibrosis including peritoneal adhesions. In general, the effect of TGF-β on tissue fibrosis occurs through increasing synthesis and deposition of extracellular matrix and decreasing their degradation through differential regulation of MMPs and TIMPs. In fibroblasts, TGF-β inhibits MMP-1, stimulates TIMP-1 expression and prevents plasmin generation by increasing the expression of plasminogen activator inhibitor (PAI-1), allowing the unopposed deposition of extracellular matrix. Fibrous adhesions and peritoneal fluid express elevated levels of TGF-β1 during the early stages of wound repair and treatment of myometrial smooth muscle cells and adhesion fibroblasts with TGF-β result in differential regulation of α1 procollagen, fibronectin, TIMP-1 and MMP-1 mRNA expression in these cells. Furthermore, TGF-β1 has been shown to suppress the expression of MMP-3 (steromlysin 1) in fibroblasts and MMP-7 (Matrilysin) in endometrial epithelial cells. It has also been reported that resting keritinocytes in normal skin do not express MMP-1 and MMP-3.

It appears that for normal healing to proceed, the expression and availability of the molecules must be optimal, precise and synchronized. Inhibition, interruption or excess expression of these molecules seem to be responsible for the failure of normal healing, either impairment (nonhealing) or excess tissue formation (adhesion development). In this regard our data provide the first evidence that an unbalanced level of MMP-1 and TIMP-1, high TIMP-1 expression, and association of a major portion of MMP-1 in complex with TIMP-1may be major contributing factors in the peritoneal environment which provide a favorable condition leading to adhesion development.

To test our hypothesis, we assessed whether MMP and TIMP expression is altered in patients who do or do not have adhesions, as well as whether there is tissue variation within the peritoneal environment which may influence likelihood of adhesions. The present study comparatively examined the expression of MMP-1, TIMP-1 and MMP-1/TIMP-1 complex in various intraperitoneal tissues including parietal peritoneum, uterus, fallopian tube, ovary, bowel, omentum and adhesions as well as in skin, fascia, and peritoneal fluids in patients who were undergoing abdominal/pelvic surgical procedures.

EXAMPLE

Tissue specimens including skin, fascia, parietal peritoneum, uterus, fallopian tube, ovary, large bowel, omentum and adhesion, as well as peritoneal fluids were collected from patients (N=55) who were undergoing abdominal/pelvic surgical procedures. Peritoneal fluids were excluded if the fluids became contaminated with blood during the collection. Thus, peritoneal fluid from 15 patients were analyzed. The collection of the tissues and peritoneal fluid from these patients was approved by the Institutional Review Board from each individual institution prior to initiation of the study. All patients gave informed written consent prior to tissue collection.

The patient's pelvic findings at surgery were used to asses the type of adhesions. The extent of adhesion formation was determined and classified based on their severity as previously described. In female patients, adhesions involving only a small area, usually the tubes and ovaries, and lysed with ease were categorized as minor, adhesions involving larger areas were classified moderate, and more vascular and cohesive adhesions were categorized as extensive. In male patients, adhesions were categorized in a similar manner, although the patients were undergoing various gastrointestinal surgical procedures.

After collection, the tissues pieces were divided into multiple portions and one portion was subjected to extraction of MMPs and TIMPs according to the protocol described in the ELISA kits and established in our laboratory. Prior to the ELISA assay, the total protein content of the tissue extracts were determined using a standard protein assay kit (Bio-Rad, Hercules CA). An equal amount of the tissue extracts and peritoneal fluids were assayed using human specific ELISA's for MMP-1, TIMP-1 and MMP-1/TIMP-1 complex with limits of detection of 1.7, 1.25 and 1.5 ng/ml, respectively, measuring the total MMP-1 (free and in complex with TIMP-1, but not with α2-macroglobulin), total TIMP-1(free and in complex with MMPs) and MMP-1/TIMP-1 complex (activated MMP-1 that has subsequently been complexed with TIMP-1). The ELISA kits were purchased from Oncogen Sciences (Cambridge MA) and used according to the procedures provided by the manufacturers. Data are expressed as mean ± SEM and significance was defined as $P<0.05$. The data were statistically analyzed using one way analysis of variance (ANOVA) and Dunn's multiple test and presented as ng of MMPs or TIMPs/ mg of total protein. Of the 55 patients, 45 were female and 10 were male, ranging in age from 24 to 83. Among the female patients, 13 were postmenopausal and 32 were premenopausal, of whom 23 had previous invasive and noninvasive pelvic surgical procedures which included cesarean sections, bilateral tubal intervention, appendectomy, ovarian cystectomy, hysterectomy and/or treatment for

endometriosis. Based on each premenopausal patient's last menstrual period and endometrial histology, 9 patients were in the proliferative phase and 23 were in the secretory phase of the menstrual cycle.

Irrespective of the patients age, gender, medical diagnosis and pervious medical history, all the tissue extracts and peritoneal fluids express MMP-1, TIMP-1 and MMP-1/TIMP-1 complex. However, the tissues and peritoneal fluids express a significantly higher TIMP-1 compared to MMP-1 or MMP-1/TIMP-1, with ranges from 2 to 10 fold higher ($P<0.05$). There were also significant variations in the levels of MMP-1, TIMP-1 and MMP-1/TIMP-1 expression in tissues and peritoneal fluid within and among the patients, ranging from 2 fold higher for MMP-1 and MMP1/TIMP-1 and up to 10 fold higher for TIMP-1 ($P<0.05$). The ovaries appeared to express a significantly higher level of MMP-1, followed by fallopian tube, large bowel, uterus, omentum, adhesion, parietal peritoneum, fascia, peritoneal fluid and skin ($P<0.001$). In contrast, the highest level of TIMP-1 expression was found in peritoneal fluid, followed by adhesions, large bowel, uterus, fallopian tube, ovary, peritoneum, omentum, skin and fascia ($P<0.01$).

In the adhesions, the level of TIMP-1 expression was substantially higher in patients with extensive adhesion, compared to moderate to mild adhesion, but was not significant. In general, the mean levels of TIMP-1, but not MMP-1 and MMP-1/TIMP-1 complex were substantially higher in all the tissues and peritoneal fluids of pre-menopausal patients compared to postmenopausal patients. Comparatively, the levels of MMP-1/TIMP-1 complex expression were similar to that of MMP-1 in the tissue extracts and peritoneal fluids, with highest level expression found in the ovary ($P<0.05$).

With respect to the type of adhesions, the peritoneal fluid of patients with extensive adhesions had a substantially higher TIMP-1. Compared to peritoneal fluid, parietal peritoneum from all patients expressed more MMP-1, but significantly lower TIMP-1 ($P<0.003$), with both expressing equal amounts of MMP1/TIMP-1 complex. Adhesions and skin expressed the lowest MMP-1 and TIMP-1 compared to other tissues. However, despite variability among the number of tissue samples, it appears that in patients with extensive adhesions, the adhesions expressed substantially more TIMP-1 than those with moderate adhesions. Essentially, most if not all the MMP-1 appears to be associated in complex with TIMP-1, both in peritoneal fluid and in all the tissues examined, ranging from 38% (fallopian tube) to 100% (peritoneal fluid).

What is claimed is:

Claims

1. A method for the prevent or remediation of surgical adhesions comprising treating a patient at risk of having such adhesions with a therapeutic formulation selected from the group consisting of antibodies to TIMP-1 and TIMP-1 antisense oligonucleotides.

2. The method of claim 1 wherein the therapeutic formulation comprises TIMP-1 antibodies.

3. The method of claim 1 wherein the therapeutic formulation comprises TIMP-1 antisense oligonucleotides.

4. An antibody to TIMP-1.

5. The antibody of claim 4 which is a monoclonal antibody.

6. The antibody of claim 4 which is a polyclonal antibody.

7. A pharmaceutical formulation comprising the antibody of claim 4.

8. The pharmaceutical formulation of claim 7 which includes a suitable carrier.

9. The pharmaceutical formulation of claim 8 wherein the carrier is hyaluronic acid.

10. A method of determining whether a human subject is predisposed to develop adhesions during or following a surgery comprising measuring the amount of TIMP-1 in the subject, and determining whether the amount of TIMP-1 is elevated or within normal ranges

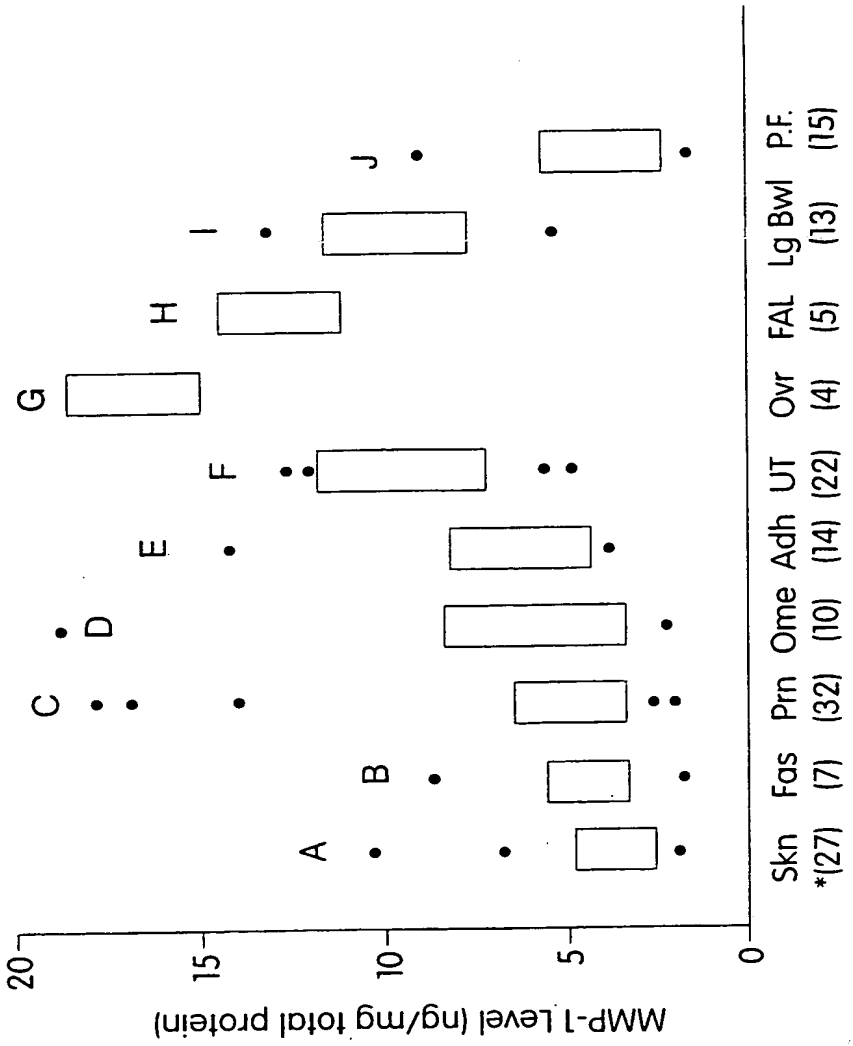
MMP-1 Level (ng/mg total protein)
20
15
10
5
0
A
B
C
D
E
F
G
H
I
J
Skn Fas Prn Ome Adh UT Ovr FAL Lg Bwl P.F.
*(27) (7) (32) (10) (14) (22) (4) (5) (13) (15)

Fig. 1

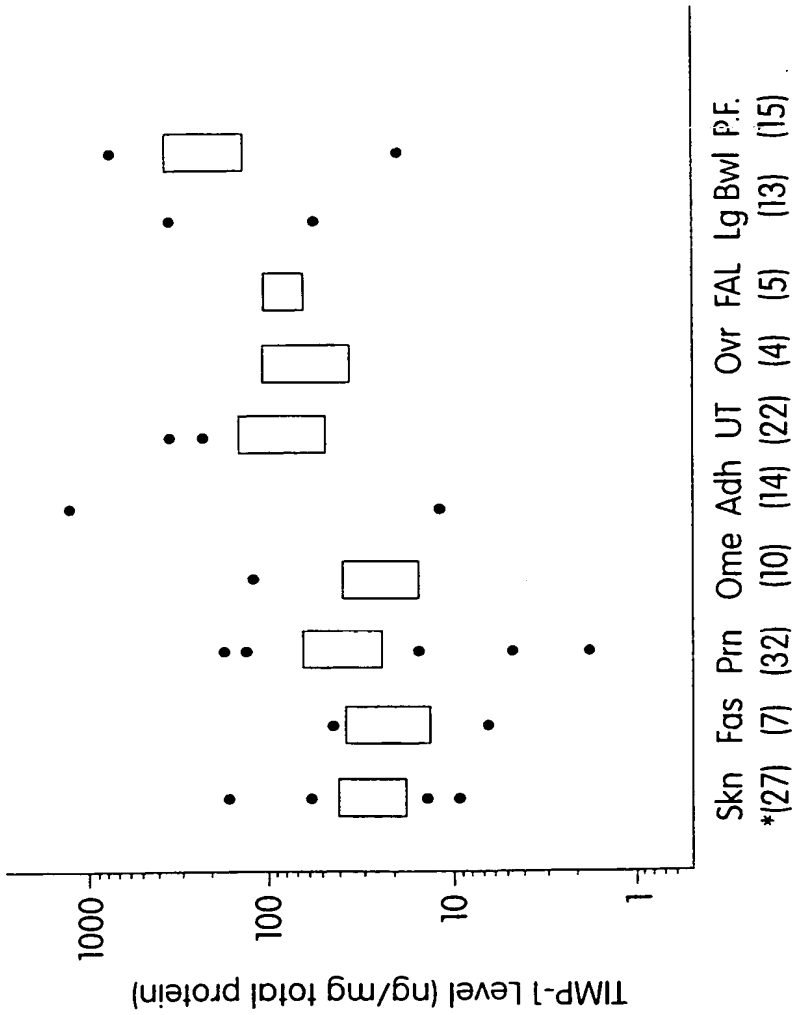
TIMP-1 Level (ng/mg total protein)
1000
100
10
1
Skn Fas Prn Ome Adh UT Ovr FAL Lg Bwl P.F.
*(27) (7) (32) (10) (14) (22) (4) (5) (13) (15)

Fig. 2

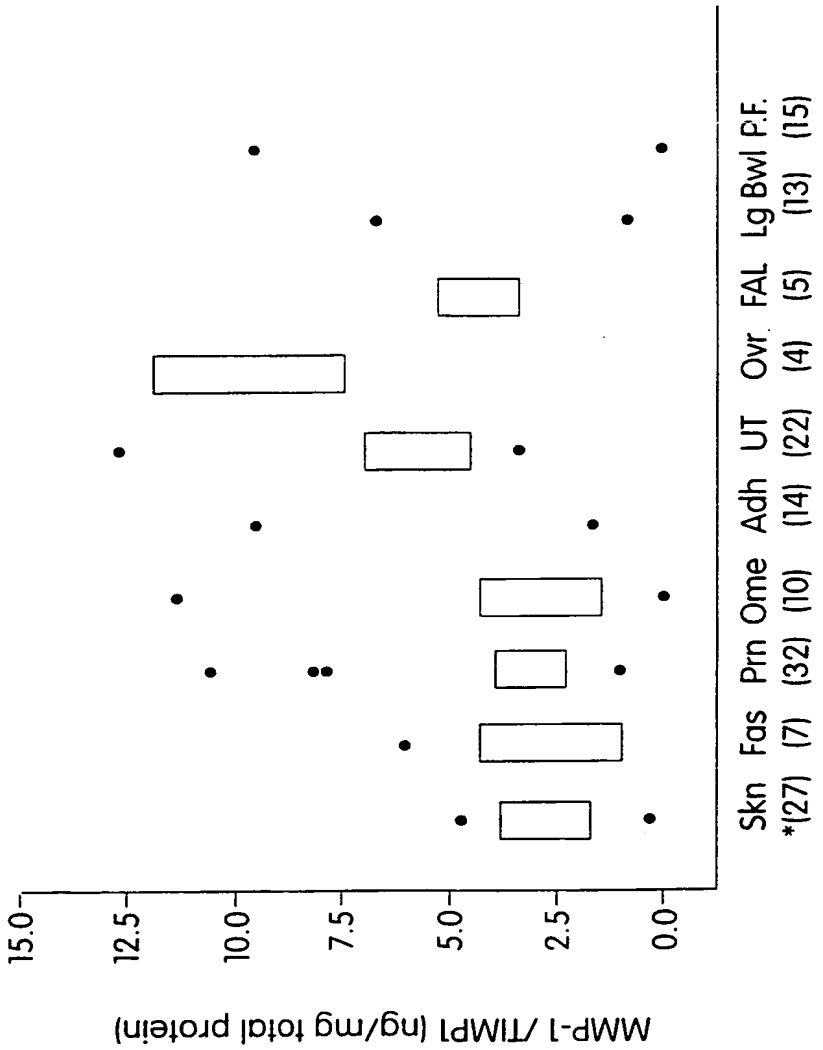
MMP-1 /TIMP1 (ng/mg total protein)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Skn Fas Prn Ome Adh UT Ovr FAL Lg Bwl P.F.
*(27) (7) (32) (10) (14) (22) (4) (5) (13) (15)

Fig. 3

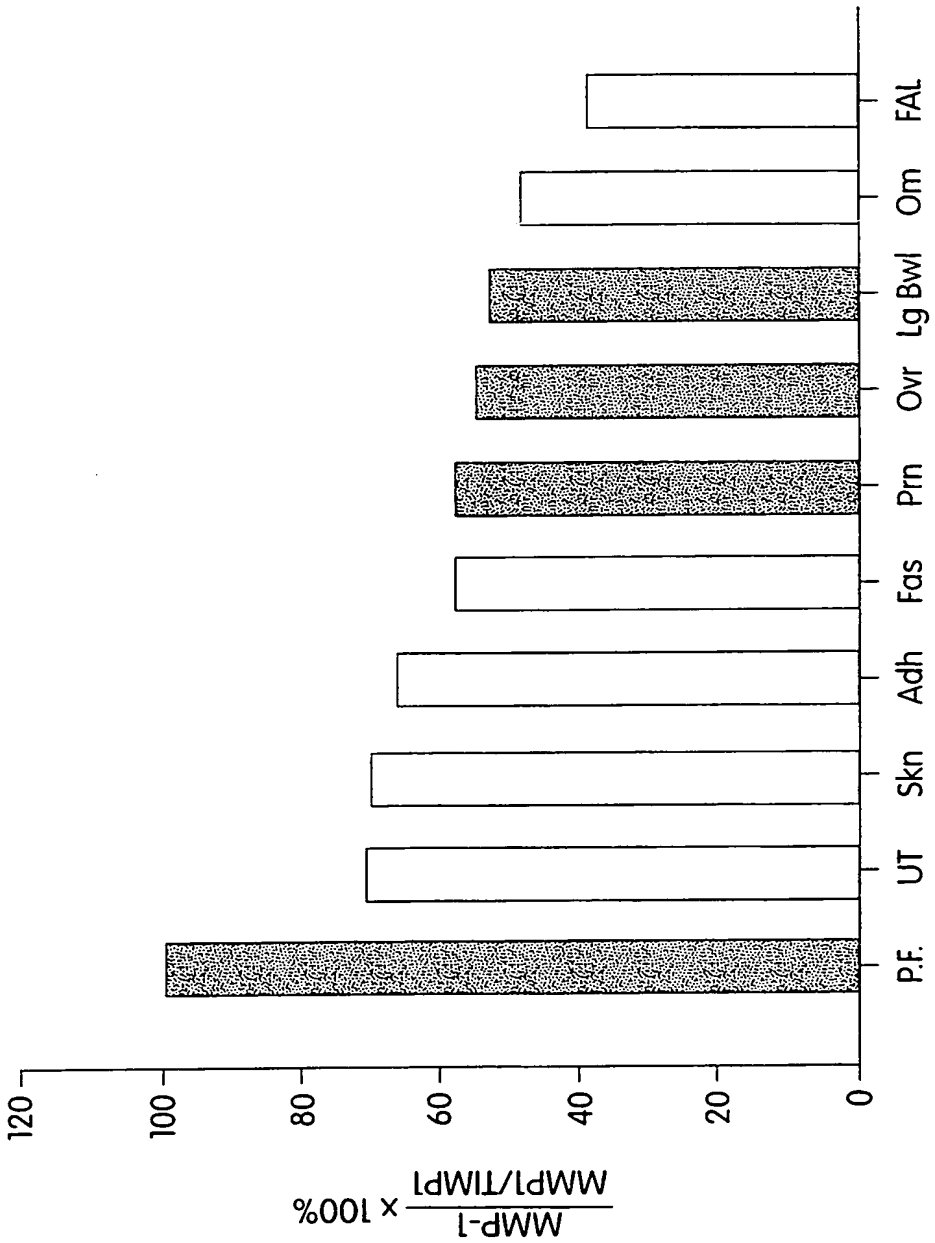
P.F.
UT
Skn
Adh
Fas
Prn
Ovr
Lg Bwl
Om
FAL
0
20
40
60
80
100
120
MMP-1 / TIMP1 × 100%

Fig. 4

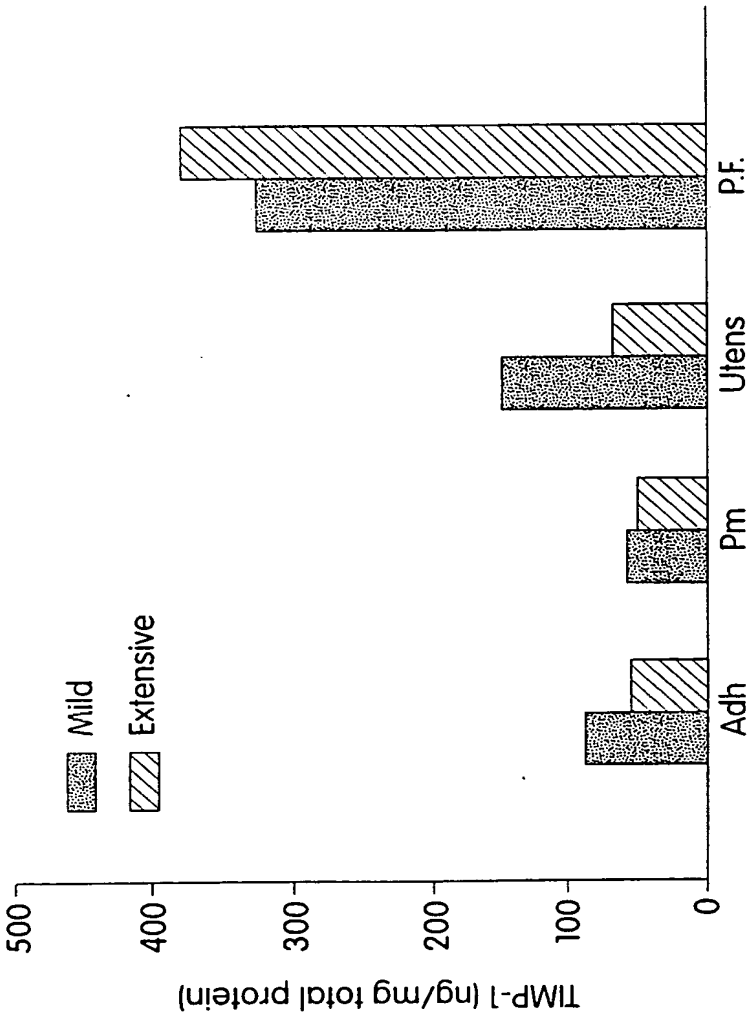
TIMP-1 (ng/mg total protein)
500
400
300
200
100
0
Mild
Extensive
Adh
Pm
Utens
P.F.

Fig. 5

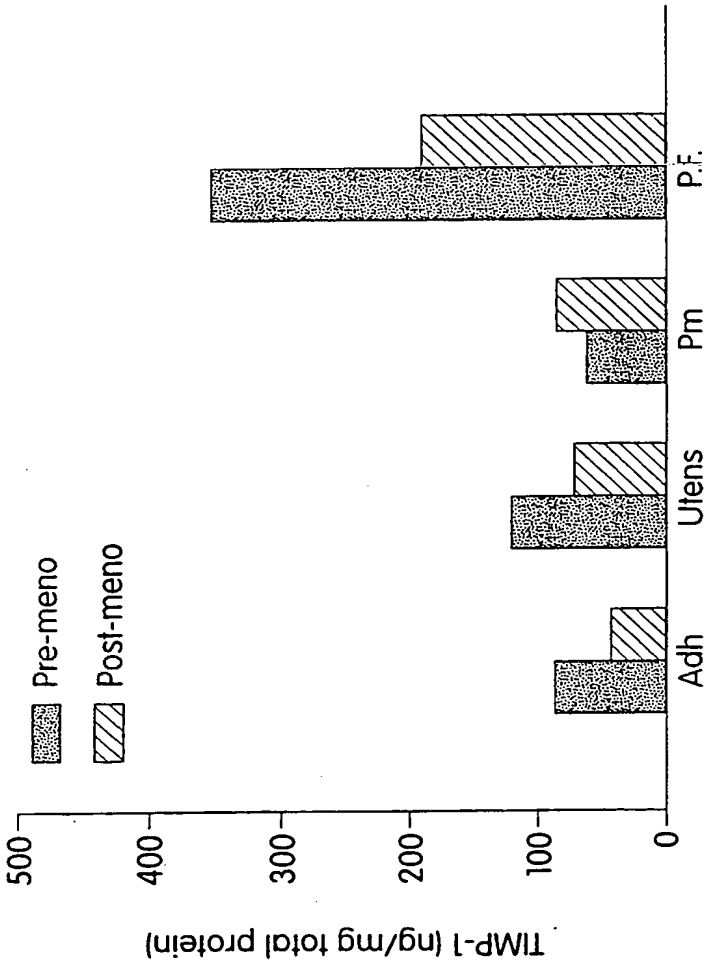
TIMP-1 (ng/mg total protein)
500
400
300
200
100
0
Pre-meno
Post-meno
Adh
Utens
Pm
P.F.

Fig. 6

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference G0651/7000WO	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US99/23014	International filing date *(day/month/year)* 01 OCTOBER 1999	(Earliest) Priority Date *(day/month/year)* 02 OCTOBER 1998
Applicant GENZYME CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is aiso accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [ ] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23014

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/68; G01N 33/53; C07K 16/44

US CL : 424/130.1, 145.1; 435/6, 7.1; 436/547; 530/387.1, 388.25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 145.1; 435/6, 7.1; 436/547; 530/387.1, 388.25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

patents, medline biosis embase caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,744,442 A (RICHARDS et al.) 28 April 1998, column 14, lines 27-30, column 20, lines 64-67, column 21, lines 1-3.	4-8 ---------- 1, 3, 9
X, P ---- Y, P	US 5,843,673 A (SHARPE-TIMMS) 01 December 1998, column 17, lines 66-67, column 18, lines 1-2, lines 42-45.	4-6 ---------- 1, 2, 7-9, 10
Y	MENINO, JR. et al. Expression of Proteinases and Proteinase Inhibitors During Embryo-Uterine Contact in the Pig. Dev. Genetics 1997, Vol. 21, pages 68-74, especially page 69 and Table on page 70.	1, 3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 DECEMBER 1999	Date of mailing of the international search report 04 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AMY DECLOUX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23014

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STEARNS et al. Cytokine (IL-10, IL-6) Induction of Tissue Inhibitor of Metalloproteinase 1 in Primary Human Prostate Tumor Cell Lines. Oncology Research. 1995, Vol. 7, Nos. 3/4, pages 173-181, especially page 178, Figure 5.	1, 3
A, E	OH et al. Matrix metalloproteinase-9/Gelatinase B is Required for Process Outgrowth by Oligodendrocytes. J. of Neuroscience. 01 October 1999, Vol. 19, pages 8464-8475.	1-10
A	HSU et al. Colon carcinoma cells with inactive *nm23* show increased motility and response to motility factors. Carcinogenesis. 1995, Vol. 16, No. 9, pages 2259-2262.	1-10